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Phase Transition of a Single Lipid Bilayer Measured by Sum-Frequency Vibrational Spectroscopy

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We report here the first use of sum-frequency generation (SFG) vibrational spectroscopy to measure the phase transition temperature (T_m) of a single planar supported lipid bilayer (PSLB) of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine (DHPC), and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) at the fused silica/D_2O interface. Unlike previous SFG studies of amphiphilic molecules at interfaces, which used changes in the number of gauche conformations in the alkyl chains to determine thermodynamic properties,[1−3] we used the inherent symmetry of the bilayer structure itself to measure the T_m. The destructive interference of the symmetric stretch (υ_s) transition moments from the fatty acid methyl groups (CH_3) is used to monitor changes in the symmetry of the bilayer structure, providing a direct measurement of the T_m. These results also provide evidence for the delocalization of domain structures between the two leaflets of lipid bilayers.

Planar supported analogues of phospholipid bilayers have been used as models for studying membrane structure and function, as biocompatible substrates for biosensors and as nonfouling protein resistant surfaces.[4−6] The efficacy of these model membrane systems has been the subject of some debate due to the lack of physical measurements which can be performed on these assemblies. Differential scanning calorimetry (DSC) can be used to measure the T_m for lipid vesicles in solution;[7,8] however, DSC lacks the sensitivity to detect the change in heat capacity of a single lipid bilayer on a planar surface. PSLBs can be deposited on a high surface area material to perform DSC measurements;[9] however, the surface morphology is often not well characterized.

SFG has been used in this study to observe the phase transition of a single PSLB on a fused silica support. SFG is a nonlinear optical spectroscopy which couples the molecular selectivity of vibrational spectroscopy (IR and Raman) with the surface specificity of a coherent second-order nonlinear optical process.[10] SFG occurs when two coherent laser beams, one visible and the other from a tunable IR laser source (ω_{vis} and ω_{IR}), are coincident on the surface. The induced nonlinear polarization at the surface results in the generation of light at the sum of the frequencies ($\omega_{sum} = \omega_{vis} + \omega_{IR}$). The symmetry constraints on SFG restrict this process to the interface, where the inversion symmetry of the bulk phases is broken, making the technique surface specific.

SFG is also inherently sensitive to the arrangement of molecular species at an interface. In particular, the terminal CH_3 groups can be used as an intrinsic probe of the symmetry of the bilayer. For a symmetric lipid bilayer, cancellation of the terminal fatty acid CH_3 υ_s transition dipoles in the upper and lower leaflets will occur, Figure 1. An increase in the membrane asymmetry will result in an increase in the intensity of CH_3 υ_s stretch as the local symmetry is relaxed.

For the SFG experiments performed here, 3 mJ/pulse of 532 nm light from a Nd/YAG laser was combined with 3 mJ/pulse of IR light (2750−3100 cm^{-1}) from an OPO/OPA (LaserVision) which was pumped with the fundamental (1064 nm) output from a nsec. Nd/YAG laser with a repetition rate of 10 Hz. PSLBs were prepared by the Langmuir−Blodgett−Schaeffer method[5] on the flat surface of an IR grade hemicylindrical fused silica prism. The lipid films were deposited at a surface pressure of 30 mN/m, which corresponds to an area per molecule of 44 ± 1.5, 45 ± 1.0, and 46 ± 1.3 Å^2 for DPPC, DHPC, and DSPC, respectively. The total sample area illuminated was ∼4 mm^2, corresponding to ∼30 pmol of lipid. The samples were transferred to a Teflon flow cell equipped with a circulating water-jacket for temperature control and a type K thermocouple with a resolution of 0.05 °C and an accuracy of 0.2 °C.

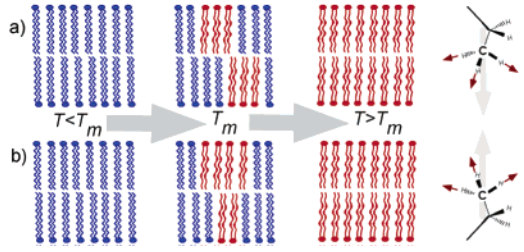

Figure 1. Representation of gel (blue) to liquid-crystalline (red) phase transition illustrating (a) domain dislocation and (b) domain size disparity which could give rise to membrane asymmetry. Also shown is the cancellation of the terminal CH_3 υ_s mode.

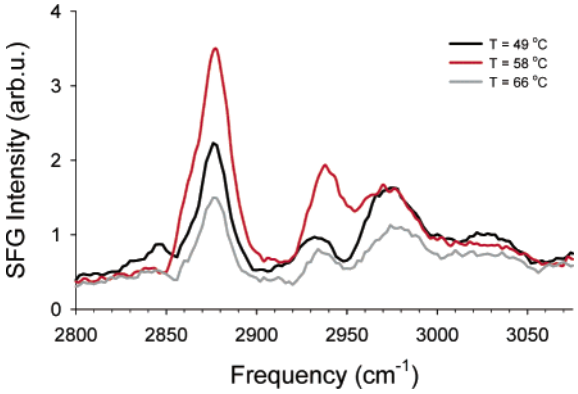

Figure 2. SFG spectra of a DSPC bilayer recorded at 49 °C, 58 °C, and 66 °C, with *s*-polarized SFG, *s*-polarized visible, and *p*-polarized IR.

Phospholipid bilayers composed of a single lipid species, such as those examined here, undergo a highly cooperative phase transition at a defined T_m.[8] Below the T_m, the lipids exist as a solidlike gel phase. Above the T_m, the lipids are in a liquid or liquid-crystalline (LC) state. The T_m is determined predominantly by the melting of the aliphatic fatty acid chains. At the T_m, both LC and gel domains, on the order of several nanometers to micrometers in size, coexist within the membrane,[11] illustrated in Figure 1.

Figure 2 shows three spectra of a DSPC bilayer recorded above, below, and at the T_m. Five CH vibrational stretching modes can be observed.[12−14] The frequencies at 2848 cm^{-1}, 2875 cm^{-1}, and 2935 cm^{-1} are assigned to the CH_2 symmetric stretch (υ_s), CH_3 υ_s, and CH_3 Fermi resonance, respectively. The peak centered at 2974 cm^{-1}

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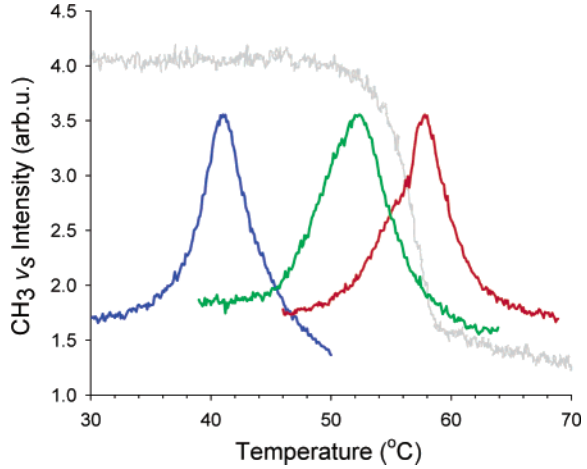

Figure 3. CH_3 vs intensity as a function of temperature for DPPC (blue), DHPC (green), DSPC (red), and a monolayer of DSPC in D_2O (gray).

is a combination band of the CH_3 antisymmetric stretch (υ_{as}) and the CH_3 υ_s from the choline headgroup.[15]

The fatty acid chains are predominantly in an all-trans conformation with some gauche defects observed in both the gel and LC phases, as indicated by the small but measurable CH_2 υ_s. There is a decrease in the CH_2 υ_s intensity with increasing temperature, which is counter to the expectation that the LC state should contain more gauche defects than the gel state. These results are consistent with previous SFG studies of lipid monolayers at the CCl_4/D_2O interface[16] but are inconsistent with IR and Raman studies of similar systems.[17−19] This discrepancy illustrates the difficulty in using the CH_2 υ_s measured with SFG to characterize the structure and T_m of lipid bilayers.

The most dynamic changes in the spectra are observed for the CH_3 υ_s at 2875 cm^{-1}. At 49 °C the lipid film is in the gel state, characterized by a well-ordered arrangement of the lipid chains. The SFG signal is weak due to the completely symmetric nature of the lipid assembly; however, a SFG spectrum is obtained, suggesting a local break in symmetry of the bilayer. This can be due to defects in the film or the fact that the "pure" symmetry of the bilayer is broken, with the lower leaflet supported on the fused silica surface and the other in contact with D_2O.

As the lipid membrane goes through a phase transition ($T = T_m$), there is a marked increase in the CH_3 υ_s intensity. A break in the local symmetry caused by dislocation of gel and LC domains in the two leaflets of a bilayer could give rise to an increase in the CH_3 υ_s resonance, Figure 1. Another possibility is that the two leaflets of the bilayer undergo the gel to LC phase transition separately, Figure 1, which has been suggested by recent DSC measurements of a PSLB on a mica surface.[21] Above the T_m, the intensity decreases due to the formation of a homogeneous LC phase, restoring the symmetry of the bilayer.

The CH_3 υ_s intensity from the fatty acid chains was also measured continuously as a function of temperature for three lipids, DSPC, DHPC, and DPPC (Figure 3). The temperature was increased at a rate of 0.2 °C per minute in each case. Maxima in the CH_3 υ_s are observed at 41.0 ± 0.4, 52.4 ± 0.7, and 57.5 ± 0.5 °C for DPPC, DHPC, and DSPC, respectively. These values correlate well with the literature T_m values of 41.3 ± 1.8, 49 ± 3, and 54.5 ± 1.5 °C for DPPC, DHPC, and DSPC, respectively.[8,20] The high degree of correlation between the SFG spectroscopic results and those obtained by differential scanning calorimetry (DSC) suggests the T_m of lipids is not significantly altered upon immobilization on a surface. The broad response observed in the temperature-dependent CH_3 υ_s signal is not due to instrumental error but rather reflects the change in membrane asymmetry as a function of temperature, suggesting that structural inhomogeneities are present before and after the T_m.

The temperature-dependent CH_3 υ_s intensity from a monolayer of DSPC in D_2O deposited on a hydrophobic silica surface (prepared by treatment with methyltrimethoxysilane) is also shown in Figure 3. Initially, there is very little change in the CH_3 υ_s intensity with temperature. Near the T_m a sharp reduction in the CH_3 υ_s intensity is observed. A similar reduction has been seen for related monolayer systems by SFG and has been attributed to an increase in the orientational disorder of the terminal methyl group.[1,2] The maximum observed in the CH_3 υ_s intensity for the DSPC bilayer correlates extremely well with the derivative of the monolayer response (56 ± 4 °C), indicating that for both systems the largest change in the SFG response is observed at the T_m. Unlike the monolayer of DSPC, the bilayer has little SFG response above and below the T_m, illustrating the cancellation effect of the CH_3 transition moments. The measured terminal methyl group orientation showed only a slight change (±8°) over the same temperature range. These results are consistent with previous IR studies[13,20] and support the conclusion that the large intensity variation observed in the pure bilayers of DPPC, DHPC, and DSPC is due to the local break in symmetry of the bilayer at the T_m and not due to changes in the tilt angle of the CH_3 group.

Although SFG is not capable of directly visualizing the types of membrane heterogeneities between the two leaflets of the bilayer which are giving rise to the increase in the CH_3 υ_s intensity, the symmetry constraints imposed on SFG lead to the conclusion that such heterogeneities must be present in the membrane in order for the changes in signal to be observed. These studies are being extended to investigate protein and small molecule interactions with membranes and the effect on T_m and membrane structure.

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Supporting Information Available: Detailed experimental description. This material is available free of charge via the Internet at http://pubs.acs.org.

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